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COMMENTARY

Parasitic helminths tip the balance: potential anti-inflammatory therapies

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Parasitic helminths are worms that are classified within the phyla Nematoda (roundworms) and Platyhelminthes (flatworms) (see Table 1). Some nematode species, Filariae being a notable example, are able to coexist with their human host for decades. Interestingly, although millions suffer severe morbidity, a lower incidence of allergy and autoimmune disease has been reported in infected individuals.[1,2] Viral and bacterial infections, and autoimmune diseases such as type I diabetes and multiple sclerosis typically induce a T helper type 1 (Th1) pro-inflammatory response producing cytokines such as interleukin-12 (IL-12), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Chronic parasitic helminth infection is associated with high levels of immunoglobulin E (IgE), eosinophilia, mastocytosis and a predominantly Th2 immune response. This Th2 response is characterized by production of IL-4, IL-5, IL-10 and IL-13,[3] a cytokine pattern that is also linked to an anti-inflammatory phenotype. There is evidence that a Th2 response is protective in the case of gastrointestinal nematodes.[4] However, there is also overwhelming evidence from other helminth infections that a Th2 response may provide an anti-inflammatory regulatory environment.[1,5] The counter-regulatory effects of Th1 and Th2 cytokines can be seen in many immune responses and there is strong evidence that the Th2 response generated by helminths can down-regulate Th1 responsiveness to other infections.

The potential for exploitation of parasite Th2 polarization is currently being investigated in systems ranging from nematodes and schistosomes to leeches and ticks. Phase I clinical trials of a parasitic helminth therapy have shown success in inflammatory bowel disease (IBD).[6] Weinstock's group used the eggs of the pig whipworm, *Trichuris suis*, in a drink formulation to treat patients with ulcerative colitis and Crohn's disease. The prevalence of IBD is increased in Western industrialized countries but is less frequent in people with blue-collar jobs who are more likely to be exposed to a dirty environment.[7] IBD, particularly Crohn's disease, is associated with a polarized Th1 inflammatory response. It therefore appears that the counter-regulatory Th2 response induced by helminths may be beneficial in this case.

The major parasite–host interaction in parasitic nematodes involves the secretion of a variety of molecules into the mammalian host environment. These molecules can be excreted or secreted and are referred to as excretory–secretory (ES) products. They have been found to contain a number of potential immunomodulatory molecules including the chemotactic inhibitors, platelet-activating factor hydrolase[8] and eotaxin-degrading protease;[9] glutathione peroxidase and superoxide dismutase;[10] phosphorylcholine (PC)-containing glycoprotein ES-62;[11] inhibitors of antigen processing, cystatins[5] and an adenosine-generating 5′ nucleotidease.[12] Homologues of transforming growth factor-β (TGF-β) and macrophage-migration inhibition factor (MIF) have been found, and other cytokine mimics, such as an IFN-γ-like molecule, have been suggested.[5]

Recently, there have been significant advances in the understanding of the molecular mechanisms involved in immunomodulation by ES products of parasitic helminths. ES-62 is a PC-containing glycoprotein released by the filarial nematode *Acanthocheilonema viteae*, which demonstrates potent immunomodulatory activity. This activity of ES-62 appears to be contained within the PC moiety covalently attached via an N-linked glycan.[13] In 1993, Harnett & Harnett[11] discovered that ES-62 was able to inhibit murine B-cell proliferation and down-regulate protein kinase C. Other *in vitro* experiments followed showing induction of anergy in Jurkat T cells,[14] differential modulation of key proliferative signalling pathways[15] and signalling of dendritic cells to acquire a phenotype driving a Th2 response.[16] More recent *in vivo* studies provided results indicating that ES-62 influences IgG subclass responses where high levels of IgG1 were produced with no IgG2a to non-PC epitopes of the molecule.[17] Production of IgG2a was restored in IL-10$^{-/-}$ mice, showing that reduction of this Th1-associated subclass is IL-10-dependent. It was also demonstrated that macrophage production of IL-12, IL-6 and TNF-α was suppressed by ES-62, contributing to an anti-inflammatory Th2 phenotype.[18] Workers from this laboratory then investigated exposure of mice to ES-62 at concentrations similar to those that might be encountered during filarial nematode infection, using surgically implanted osmotic pumps. Purified splenic B cells recovered from these mice were hyporesponsive.[19] However, murine

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Table 1. Major parasitic helminths of humans

Helminths	Disease
Nematodes	
Wuchereria bancrofti, *Brugia malayi*	lymphatic filariasis, elephantiasis
Onchocerca volvulus	onchocerciasis (river blindness)
Ascaris lumbricoides	gastrointestinal complications, anaemia, malnutrition
Trichinella spiralis, *Ancylostoma duodenale*, *Necator americanus*	
Platyhelminths	
Trematodes (flukes)	
Schistosoma mansoni	schistosomiasis (bilharzia)
Cestodes (tapeworms)	
Taenia saginata	cysticercosis, taeniasis

peritoneal B1 cells, which are T-cell independent and produce IgM to non-peptide antigens such as PC, were activated.[20] This is particularly interesting as anti-PC IgM has been found in humans with filariasis. The question of whether ES-62 could be considered as a therapeutic agent was addressed in studies by McInnes *et al.*[21] using a murine model of rheumatoid arthritis. ES-62 was able to suppress collagen-induced arthritis if it was given during collagen priming, and importantly, if it was given after the onset of disease. These results clearly offer hope of a novel anti-inflammatory therapy.

In the current issue of *Immunology*, the article by Goodridge *et al.*[22] presents compelling evidence that the PC-containing glycoprotein ES-62 can subvert the development of dendritic cells in the bone marrow. Dendritic cells and macrophages, derived *ex vivo* from bone marrow cells exposed to ES-62 via osmotic pump, were hyporesponsive to lipopolysaccharide. This was in terms of pro-inflammatory cytokine and costimulatory molecule expression. It was also demonstrated that the PC moiety attached to an irrelevant protein could mimic the effects of ES-62. PC is a common pathogen-associated molecular pattern, and appears to be responsible for many of the immunomodulatory properties of ES-62. The polarizing effect of ES-62 *in vivo* is intriguing as it opens up the possibility of interaction with pattern recognition receptors such as Toll-like receptors.

Another potential immunomodulatory ES product has been characterized by Gounaris *et al.*[12] This group demonstrated that the nematode *Trichinella spiralis* secretes a 5′ nucleotidase, which hydrolyses AMP to adenosine. This purine nucleoside is produced at sites of inflammation and binds to P1 purinergic receptors on all immune cells. It is well known that adenosine has a central role in regulation and limitation of tissue damage, endowing it with anti-inflammatory properties.[23] Although adenosine is normally present at concentrations <1 μM around unstressed tissues, patients with rheumatoid arthritis can have adenosine concentrations of 10–100 μM in their synovial fluid. In response to stress it appears that adenosine is produced as a 'retaliatory metabolite'[24] to protect tissues. Inflammation is likely to be initiated as *T. spiralis* migrates through the intestinal epithelium. But what better way to prevent damaging inflammation than to secrete your own adenosine-generating enzyme? It would seem that a derivative of adenosine may be used as an anti-inflammatory drug in the not too distant future. However, a word of caution: because of the non-selective nature of adenosine and the expression of adenosine receptors on most cell types, the design of highly specific drugs would be required. It is also important to note that in patients suffering from asthma and chronic obstructive pulmonary diseases high levels of adenosine are already detected.[24]

One of the most striking markers of inflammation is the recruitment of inflammatory cells, in particular neutrophils. Neutrophils normally arrive at sites of injury and inflammation within a few hours to kill invading pathogens and to remove debris. However, in chronic disease states, e.g. cystic fibrosis, neutrophils can contribute to significant immunopathology. Parasitic helminth infections are associated with eosinophilia; however, neutrophils are less prevalent. Neutrophils have the potential to kill nematode larvae.[25] However, low numbers of this cell type in the lung airways have been noted following infection with *Nippostrongylus brasiliensis*[26] and with *Trichinella spiralis*.[27] There is some evidence that parasitic nematodes may be able to inhibit neutrophil recruitment. Keir *et al.*[28] showed that ES products from *N. brasiliensis* L3 infective stage larvae, instilled into the rat lung, reduced neutrophil numbers against a background of lipopolysaccharide inflammation. The mechanism of inhibition is unclear, however, a similar molecule to the neutrophil inhibitory factor of *Ancylostoma caninum*, which interferes with the integrin CD11b/18,[29] is a possibility.

Interesting new research highlights the importance of Th2-promoting nematode-elicited macrophages (NeMϕs).[30,31] These are similar to alternatively activated macrophages, in the anti-inflammatory response. Alternatively activated macrophages differ from classically activated macrophages in that their function is primarily in the resolution of inflammation. NeMϕs produce arginase rather than the classical inducible nitric oxide synthase (iNOS), depleting classical macrophages of the arginine substrate for NO. NeMϕs are cytostatic, by a contact-dependent mechanism, but this is independent of NO, IL-4, IL-10, TGF-β and prostaglandins. These cells also produce two new genes, YM1 and FIZZ1,[32] where YM1 may be responsible for the attraction of eosinophils. This system provides an opportunity to examine the function of alternatively activated macrophages, and the anti-inflammatory response *in vivo*.

In summary, studies like those of Goodridge *et al.*[22] provide important information to extend our understanding of the generation of anti-inflammatory responses. It is perhaps ironic that we return to helminths, experts in subversion of the immune response, to provide answers for the treatment of inflammatory disease.

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